ACCUGEN LABORATORIES, INC.

PROTOCOL

Confirmatory Efficacy Data- -AOAC Fungicidal Activity Test

TEST ORGANISMS
Trichophyton mentagrophyte

STUDY REQUIREMNETS
CFR 40 part 160

AUTHOR
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SPONSOR
HSP USA LLC
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TESTING LAB

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Date 04-03-13

Title:

AOAC Fungicidal Activity of Disinfectants

Objective:

To confirm the evaluation of the potential of the test agent to disinfect surfaces, the test is designed to substantiate confirmation of the disinfectant effectiveness claim for a product to be registered with the US Environmental Protection Agency. It measures the potential of the test agent to disinfect hard surfaces contaminated with fungi. The test follows Official Methods of Analysis, Eighteenth edition, 2010, AOAC , required by EPA DIS/TSS- 6.

Test Method Summary:

It follows the Use-dilution methodology used to determine the efficacy of disinfectants against fungi on hard surfaces based on AOAC methods 955.17 (Fungicidal Activity of Disinfectant Test). One sample representing one lot of the test agent, will be tested. A total of ten carriers per lot of test agent will be evaluated for efficacy against Trichophyton mentagrophyte (T.mentagrophyte, ATCC No. 9533). Cultures will be dried on stainless steel penicylinders which will be exposed to the test agent at the temperature and time stipulated by the sponsor. The carriers will be removed from the test agent, neutralized and cultured in appropriate growth media for at least ten days at 25-30^{0}C, then observed for visible growth.

Materials:

A. Test agents supplied by the sponsor: see last page.

The test agent will be tested as supplied by the sponsor unless directed otherwise. All operations performed on the test agent such as dilution or specialized storage conditions must by specified by the sponsor prior to the initiation of testing.

The sponsor assures ACCUGEN LABORATORIES, INC. that the test agent has been tested for identity, strength, stability and purity.

ACCUGEN will retain all unused test agents for a period of three months after completion of the test.

B. Materials supplied by ACCUGEN LABS, INC., including, but not limited to:

1. Challenge fungus required by AOAC, EPA -*Trichophyton mentagrophytes*, ATCC 9533.
2. Media and reagents:
    a. Sterile saline solution (SS).
    b. Neopeptone Glucose Broth (NGB).

c. Neopeptone Glucose Broth with neutralizers (NGB+).

d. Neopeptone Glucose Agar (NGA).

3. Laboratory equipment and supplies

Carriers. Polished stainless steel cylinders

Glassware.

25 × 100 mm tubes For disinfectant.

20 × 150 mm tubes For cultures/subcultures

16 × 100 mm screw cap tubes For stock cultures.

All glassware will be steam sterilize for a minimum of 20 minutes at 121°C with drying cycle.

Water bath. Constant temperature for test chemical, capable of maintaining 20 ± 1°C temperature or specified temperature for conducting the test.

Incubator

Test tube racks.

Transfer loops.

Timer.

Microscope

All Petri dishes and dilution tube racks will be labeled with microorganism, test agent and project number.

Procedure:

A. Inoculum preparation:

The fungus will be inoculated from the stock culture onto NGA plates and incubated at 25 – 30 ^{0}C for 10 to 15 days. When the cultures appear to be mature, the mycelial mats will be removed from the surface of at least five plates and macerated with sterile saline in a sterile glass tissue grinder. The suspension will be filtered through sterile glass wool to remove the hyphae.The density of the conidial suspension will be determined by serially diluting the prepared culture in saline Aliquots from selected dilution will be plated on duplicate NGA plates. The plates will be incubated for 3-5 days at 25-30^{0}C. The suspensions will be stored at 2 – 10^{0} C for 4 weeks before use. The cultures will be standardized to yield dried carrier counts of 1 x 10^{4} to 1 X 10^{5} cfu per carrier.

B. Carrier preparation:

Carriers will be washed with water . Ethyl alcohol will be added and decanted and the carriers will be rinsed three times with deionized water. Cleaned carriers will be covered with water, steam-sterilized, cooled and stored at room temperature until use. The water will be decanted aseptically from the prepared carriers and any water remaining on the bottom will be removed with a sterile

pipet. Twenty eight mL of the standardized culture (1 mL for each of the 28 carriers) will be added to the tube and held for 15 min at room temperature. The carriers will be removed from the suspension and placed into sterile Petri dishes lined with filter paper. Each carrier will be tipped to 60°; rotated 360° to blot off excess medium and then moved to a dry section of paper standing upright without touching. The inside of each carrier will be probed with a Dacron swab to removed excess liquid from the inside of the carrier. One swab will be used for 12 carriers before it is discarded. The carriers will be dried in a dry incubator for 40 min at 30°C.

C. Preparation of test agent:

The test agent will be prepared according to the sponsor's specifications and dispensed in 10-mL aliquots into sterile test tubes. The tubes will be placed in a water bath and allowed to come to test temperature for at least ten minutes before testing.

If requested by sponsor of study, 5% serum will be added as organic load to each lot of test material.

D. Test:

Tubes containing the test agent will be maintained at testing temperature throughout the test. One contaminated carrier will be added to each tube; the tube swirled to mix; and the carrier allowed remaining in contact with the test agent for a time specified by the sponsor of the study. After the contact time, the carriers will be removed, transferred to recovery broth with neutralizers (ten mL of NGB+ tubes). and the tubes will be thoroughly shaken. After 30 minutes, the carriers will be transferred to secondary subculture(NGB+) tubes. This process will be repeated for all tubes All tubes will be incubated for at least 10 days at 25-30°C and the results will be recorded as visible growth or no visible growth.

E. Controls:

1. Sterility controls:

One tube of recovery broth with neutralizers containing a single sterile carrier will be incubated with the test.

2. Neutralizer effectiveness and toxicity:

A test tube containing ten mL of the test agent will be allowed to equilibrate to testing temperature for at least 10 min. A single sterile carrier will be added to the tube and held for the same time as the test carriers. After the contact time, the carrier will be added to a tube containing recovery broth with neutralizers and fewer than 100 CFU of the challenge microorganism will be added to the tube. The CFU added to the tube will be confirmed.

All tubes and plates will be incubated with the test.

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3. Carrier counts:

The average CFU per carrier will be determined using three carriers. Dried carriers will be placed individually into tubes containing 10 mL PBDW containing 1% Polysorbate 80. Serial tenfold dilutions of each suspension will be performed in PBDW blanks.

Duplicate one-mL aliquots from selected dilutions will be plated in Neopeptone Glucose Agar pour plates. All plates will be incubated with the test and the average CFU/carrier determined.

4. Viability controls:

Two inoculated carriers will be inoculated into tubes of recovery broth with neutralizers and incubated with the test to serve as comparison for the test cultures.

5. Confirmation of challenge microorganisms:

Samples from tubes showing growth will be examined using wet mount

Product Evaluation Criteria:

According to EPA guidelines the test material meets the effectiveness requirements, if no growth occurs in all ten test tubes per lot. All fungal spores on all 10 carriers should be killed within ten minutes or less.

Data Presentation:

The final report will include the following information in tabular form if appropriate:

• The number of positive tubes per lot of test material.

Test Acceptance Criteria:

The test will be acceptable for evaluation of the test results if the criteria listed below are satisfied. The study director may consider other causes that may affect test reliability and acceptance. There are no proposed statistical methods for this test.

• All growth from positive tubes will be identified to confirm that the culture is indeed the challenge fungus.

• The positive control must exhibit growth of the challenge fungus.

Report Format:

ACCUGEN LABS employs a standard report format for each assay design. Final reports provide the following information:

• Sponsor identification

- Test material identification
- Type of assay and project number
- Dates of study initiation and completion
- Interpretation of results and conclusions
- Signatures of responsible personnel
- Test results presented in tabular form
- Methods and evaluation criteria
- Quality Assurance and Compliance Statements

Records to be maintained:

All raw data, protocol, protocol modifications, test agent records, final report, and correspondence between ACCUGEN LABS and the sponsor will be stored in the archives at ACCUGEN LABS, INC. All changes or revisions to this approved protocol will be documented, signed by the study director, dated and maintained with this protocol. The sponsor will be notified of any change, resolution, and impact on the study as soon as practical.

Personnel and Testing Facility:

A study director will be assigned before initiation of the test. This study will be conducted at ACCUGEN LABS, INC., 50 West, 75th street, Willowbrook, IL 60527.

REFERENCE:

Official Methods of Analysis. July 2012, 18th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, (Methods 955.17).

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MISCELLANEOUS INFORMATION:
The following information is to be completed by sponsor before initiation of study:

A. Name and address: HSP USA LLC
3111 Route 38, Suite 11
Mount Laurel, NJ 08054

B. Test agent Name: (H_2O_2)

Lot No: 518

Product description: ☐ Sodium hypochlorite ☐ Quatenary ammonia ☐ Iodophore
☐ Peracetic acid ☐ Peroxide ☒ Other Hypochlorous Acid

Active ingredients: ↙

Test Agent Preparation:

☒ Ready to use, no dilution ☐ Dilution/ Concentration to be tested_____

If need to be diluted-
Diluent ☐ Deinized water ☐ tape water ☐ Hard water ____ppm
☐ Other_____

C. Contact Time: One minute

D. Contact Teperature: 20°C

E. Organic Soil ☐ Yes ☒ N/A

F. Precautions/storage conditions ☒ Room Temperature ☐ 2-8 C ☐ Other AWAY FROM LIGHT / HEAT

MSDS or Certificate of Analysis ☐ Provided ☒ Not provided

REPORT HANDLING The sponsor intends to submit this information to
☒ US EPA ☐ US FDA ☐ Health Canada ☐ ARTG
☐ GLP ☐ non-GLP ☐ DPR ☐ other

PROTOCOL APPROVAL

For
Accugen Laboratories, Inc..

Signature
TEHSEEN NAQVI, Study director
Name and Title
5/8/13
Date

For
HSP USA

Signature
HENRY DAO, CEO
Name and Title
5/7/13
Date